Atty. Dkt. No. 065334-0110

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Iacob MATHIESEN et al.

Title: ELECTROPORATION DEVICE AND INJECTION APPARATUS

Appl. No.: 10/612,304

Filing Date: 07/03/2003

CLAIM FOR CONVENTION PRIORITY

Commissioner for Patents
PO Box 1450
Alexandria, Virginia 22313-1450

Sir:

The benefit of the filing date of the following prior foreign application filed in the following foreign country is hereby requested, and the right of priority provided in 35 U.S.C. § 119 is hereby claimed.

In support of this claim, filed herewith is a certified copy of said original foreign applications:

- GB 0215529.9, filed July 4, 2002; and.
- GB 0215523.2, filed July 4, 2002.

Respectfully submitted,

Date 3 November 2003

By S. A. Bent

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P01/7700 0.00-0215523.2

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04 JUL 2002

The Patent Office
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1.	Your reference	15.76879
2.	Patent application number *(The Patent Office will fill in this part)*	0215523.2
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	Inovio AS Oslo Research Park Gaustadalleen 21 N-0349 Oslo Norway
	Patents ADP number *(if you know it)*	84187201
	If the applicant is a corporate body, give country/state of incorporation	Norway
4.	Title of the invention	Electroporation Device
5.	Name of your agent *(if you have one)*	Frank B. Dehn & Co.
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	179 Queen Victoria Street London EC4V 4EL
	Patents ADP number *(if you know it)*	166001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number	Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
   a) any applicant named in part 3 is not an inventor, or
   b) there is an inventor who is not named as an applicant, or
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Yes

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Continuation sheets of this form	-
Description	11
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Priority documents	-
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Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	-
Request for preliminary examination and search *(Patents Form 9/77)*	-
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Any other documents *(please specify)*	-

11. I/We request the grant of a patent on the basis of this application.

Signature Philippa Power Date 4 July 2002

12. Name and daytime telephone number of person to contact in the United Kingdom

Philippa Power
020 7206 0600

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Electroporation Device

The present invention relates to electroporation, i.e. the process of introducing substances into cells during or after the application of an electric field. More particularly, the present invention relates to a device for use in electroporation.

Electroporation is used for example in the treatment of cancer or in gene therapy. Electroporation provides a method of delivering pharmaceuticals or nucleic acids (e.g. DNA) into cells, e.g. skeletal muscle cells. Thus for example the muscle may be electrically stimulated at the same time or shortly after the pharmaceutical or DNA is injected. This method works on the principle that cells act as an electrical capacitor generally unable to pass current. Subjecting the cells to an electric field creates transient permeable structures or micropores in the cell membrane. These pores are large enough to allow the pharmaceuticals and/or DNA to gain access to the cells. With time, the pores in the cell membrane close and the cell once again becomes impermeable.

Various devices for effecting electroporation have been suggested. US6,208,893 discloses an electrode template apparatus having a plurality of bores through which a plurality of needle electrodes extend, each bore being separately connected to a conductor so that each of the electrodes can be connected to a power supply in use. An insulating portion can be provided along the midportion of each electrode so as to isolate the body tissue adjacent the insulated part of the needle from the electric field produced by the electrode in use. Further, one or more of the needle electrodes may be hollow and can include openings through which medicinal substances can be injected into the body tissue.

EP0693951B discloses a device for the implementation of electrochemotherapy. The device comprises electrode needles through which electric pulses are applied. The electrode needles are hollow so as to allow active substances to be injected locally into the body tissue to be treated. Holes can be provided along the length of the needles as well as at the ends thereof to improve the distribution of injected substances. An insulating sheath can also be provided over a part of the needle lengths as a means of preventing the application of electrical pulses to certain zones.

The present invention seeks to provide a device which can be used in electroporation in vivo, in particular in gene therapy.

Gene therapy by electroporation involves administering a dose of between about 10 μL and 1 ml of DNA solution. DNA is toxic if too much is incorporated into cells and so the quantity of DNA in solution must not be too high. Thus, the quantities of solution are relatively small and, especially in larger animals such as human beings, it is difficult to administer both DNA and electric field to the right place in the muscle. Further, as the cells being treated should not be damaged, the electroporation device should be much gentler than the prior art devices whose primary use is in the treatment of cancer where the treated cells are killed. Ideally therefore, the electroporation device should not produce undue fields due to edge effects on the electrode thereof and should also not include any relatively blunt or bulky tissue piercers.

From a first aspect, the present invention provides an electroporation device comprising: a needle for injecting a substance into body tissue; and an insulating sheath adapted to surround the needle and

having one or more apertures formed along the length thereof through which the electric field may propagate in use, wherein the needle is axially moveable relative to the sheath.

The device of the invention has the advantage that if the needle is also used as an electrode, as the needle is axially moveable relative to the sheath, the needle can be withdrawn so that the insulating sheath completely surrounds the needle after the device has been inserted into the body tissue and before the electric field generating means are activated. Thus in use, the electric field propagates through the apertures in the sheath, and so the formation of uneven electric field strengths in the body tissue to be treated is avoided as no edge effects are created.

Preferably, the needle for injecting a substance into body tissue also constitutes an electrode via which an electric field is propagated in use. Thus, in this preferred embodiment, the needle is connectable to a voltage source. It will of course be appreciated that, in one embodiment, the needle could remain connected to the voltage source at all times.

However if necessary, the device may be adapted to allow the needle to be removed from the insulating sheath after injection of the substance into the body tissue so that the needle can be replaced by an electrode rod prior to activation of the electric field. This would be advantageous for example to avoid the release of unwanted metal ions by the needle which could be caused by the provision of an electric charge on the needle. In this embodiment, the electrode rod would be arranged so as to be completely surrounded by the sheath in use so that again, no edge effects would be produced by the electric field in use.

The sheath could be formed of any electrically insulating and biologically compatible material. Preferably however, the sheath is formed from polytetrafluoroethylene (Teflon^{RTM}).

Any number of apertures could be provided in the insulating sheath. In one preferred embodiment, the apertures are provided along one axially extending line on the sheath only. In an alternative preferred embodiment, the apertures are provided so as to be spaced around the circumference of the sheath. The actual number and arrangement of apertures provided in the sheath will depend on the electric field patterns required in the tissue to be treated.

The apertures in the insulating sheath could be formed in a number of ways such as but not limited to: cutting through the sheath, pushing the apertures out or laser ablation. Where apertures are required on one side only of the sheath, during aperture formation a rod can be provided within the sheath to prevent holes forming on both sides.

The electroporation device of the invention could be used alone. Preferably however, two electroporation devices are used together and if required, any number of the devices could be used. Thus for example, a group of four, six or eight devices could be used. Where one or more devices are used, the needles and sheaths can be mounted to extend downwardly through a block in which they are arranged adjacent to one another.

Preferably, means are provided such that in use the depth of insertion of a needle is determined and injection of a substance into the body tissue to be treated is commenced when the needle has reached a desired depth.

This is believed to be novel and inventive in its own right and so from a further aspect the present invention provides a device comprising a needle for injection of a substance into body tissue, and means for sensing the depth of insertion of the needle and commencing injection of a substance via the needle when a desired depth has been reached.

Various means could be provided to determine that the needle has reached a desired depth for injection to commence. For example, means for determining the electrical resistance of the tissue which will vary depending ontissue type (dermis, fat or tissue) could be provided. Preferably however, a moveable contact can be provided on the device such that in use, the contact determines when the needle has been inserted to a sufficient depth into the body tissue to be treated and then causes injection of a substance to commence. This allows automatic injection of a substance to commence when the needle reaches the correct depth in the body tissue to be treated. The injection can be carried out either while the needle is stationary or while it is continuing to be inserted.

Still more preferably, the moveable contact further determines when the needle has been inserted to the maximum depth at which injection should be carried out and then causes injection of the substance to stop. In this way it is possible for the substance to be automatically injected over the height of tissue over which an electric field will be produced in use.

Viewed from a further aspect the invention provides a method of electroporetic treatment of a human or non-human animal (e.g. a mammal, bird or reptile), said method comprising inserting the needle of a device according to the invention into tissue (e.g. muscle

tissue) in said animal, injecting an active agent (e.g. a pharmaceutical or nucleic acid) through the needle into the tissue, withdrawing the needle such that the tip thereof is within the sheath, and applying an electric field between the needle and an electrode.

It will be appreciated that the electrode could be provided by the needle of a second device according to the invention disposed inside a further sheath. Alternatively, the electrode could be a different type of electrode which had been inserted into the body tissue or an electrode which had been applied to the skin surface.

Viewed from a still further aspect the invention provides a method of electroporetic treatment of a human or non-human animal (e.g. a mammal, bird or reptile), said method comprising inserting the needle of a device according to the invention into tissue (e.g. muscle tissue) in the animal, injecting an active agent (e.g. a pharmaceutical or nucleic acid) through the needle into the tissue, withdrawing the needle from the sheath, inserting a first electrode into the sheath such that the tip of the first electrode does not extend out of the sheath into the tissue, and applying an electric field between the first electrode and a second electrode.

It will be appreciated that the second electrode could be provided by the needle of a second device according to the invention disposed inside a sheath. Alternatively, the electrode could be a different type of electrode which had been inserted into the body tissue or an electrode which had been applied to the skin surface.

The device according to the invention could for example

be used in the method of WO98/43702, the contents of which are herein incorporated by reference. Preferably, the device would be used with a square bipolar electric pulse.

A preferred embodiment of the invention will now be described, by way of example only, and with reference to the accompanying drawings in which:

Figure 1 is a schematic side elevation view of an electroporation device according to the invention; and

Figures 2a to 2c are schematic side elevation views showing three stages in the operation of an electroporation device including a skin contact device according to the invention.

As shown in Figure 1, the electroporation device according to one embodiment of the invention comprises two separate needle assemblies 2 mounted adjacent to one another in a support block 4. Each needle assembly 2 comprises a needle 6 having a sharp end 8 which is open to allow the injection of fluids via the opening. The other end of each of the needles 6 is connected to a fluid holding chamber 10 having a piston 12 arranged therein so as to form a syringe arrangement for injecting fluid via the needles in use.

First and second electrically insulating sheaths 14 made of Teflon^{RTM} and having a greater cross sectional diameter than that of the needles 6 are arranged to extend around the needles 6. Three apertures 16 spaced apart in the axial direction are provided along the length of each sheath 14. The device is configured so as to allow axial movement of the needles 6 relative to the sheaths 14.

A voltage supply 18 is provided on the support block 4 which can be connected and disconnected from the needles 6 of the electroporation device.

In use, a required dose of DNA (which could for example be 100μL) is provided in each of the fluid holding chambers 10 and the needles 6 are inserted into the skin of an animal or person to be treated. It is advantageous that the volume of fluid for injection should be small as this will insure that the injected fluid is kept close to the shaft of the needle (i.e. will be kept within a high electric field strength zone during electroporation). At this stage, the sharp ends 8 of the needles 6 extend beyond the Teflon sheaths 14 and so provide a sharp surface for piercing the skin and penetrating into the muscle or body tissue to be treated. During insertion, the relative position of the needles 6, sheaths 14 and support block 4 does not vary as the elements are locked into place relative to one another. The needles are then inserted further until they reach the correct depth in the muscle or other body tissue to be treated. Once they have reached this depth and while still being inserted, the DNA is injected into the muscle by pushing downwardly on the pistons 12 to empty the fluid holding chambers 10. If necessary, the needles can then be pushed further down into the muscle after injection. This ensures that the needles acting as electrodes cover the area into which the fluid has been injected.

After insertion of the needles and once the DNA has been injected, the needles 6 are withdrawn slightly (i.e. moved axially towards the support block 4) relative to the Teflon sheaths 14 which remain in their original position. Thus, the sharp ends 8 of the needles 6 are retracted to locate within the Teflon sheaths 14. Once the needles 6 have been retracted as described, the

voltage source 18 is activated and electroporation proceeds with each of the needles 6 acting as an electrode. The electric field produced by the electrode needles 6 propagates into the muscle or body tissue to be treated via the apertures 16 formed along the length of the Teflon shields 14. This has the advantage that no unwanted edge effects are created in the muscle or body tissue to be treated.

In a further improvement to the device of Figure 1, means are provided to sense when the needles 6 are at the correct depth in the muscle or body tissue for injection of the DNA to begin and to automatically move the pistons 12 to effect the injection. These means comprise a moveable skin contact 20 which contacts the skin S as shown in Figures 2a to c. As the needles 6 are inserted into the muscle or body tissue to be treated, the contact 20 is pushed upwardly towards the support member 4. The contact member 20 is attached to a lever mechanism consisting of a substantially vertical link 22 extending upwardly from the contact member 20 and a lever 24 which is attached at a first end to the vertical link 22. The lever 24 is attached at its other end to means 26 for causing the pistons 12 to move downwardly. The lever is adapted to pivot about a point 28 located between the two ends of the lever 24. Thus, as the contact 20 moves upwardly relative to the support member 4 in use, the lever 24 pivots causing the piston moving means 26 to push the pistons down gradually so as to effect injection of the fluids over the height of the needles being inserted. As shown, the piston moving means comprise a vertical member 27 attached to the lever 24 to move downwardly as the lever pivots and a cross piece 30 attached to the other end of vertical member 27 which acts to push the pistons down as it moves downwardly with the vertical member.

The relative location of the skin contact 20 and lever mechanism can be adjusted to ensure injection of the fluids once the needles have reached the muscle tissue and while they are being inserted further into the tissue to ensure a uniform distribution of sample in the area around the electrodes in the muscle.

Figure 2a shows the device before the pistons have been pushed down with the tips of the needles only inserted into the skin. Figure 2b shows the device when the needles are fully inserted to the required depth in the muscle tissue and the pistons 12 have been fully depressed by the action of the lever mechanism. Figure 2c shows the device once the needles have been attached to a power supply 18 after injection of the fluids. As shown, the syringes have been removed although this is not essential.

In alternative embodiments, lasers or sensors could be used to detect the depth of insertion of the needles and automatically initiate injection of the fluids at a desired depth instead of the mechanical skin contact arrangement described above.

The contact or sensors can be further adapted to sense when the needles 6 have reached a depth in the body tissue at which injection of the fluids should stop so as to ensure that fluid is only injected into the height of body tissue to which an electric field will be applied in use.

It will be appreciated that one advantage of the embodiment of the invention described above is that known cannula devices which are already on the market and so have marketing approval can be used to provide the needle and sheath assemblies of the device, the only modification which is required being the formation of

the apertures 16 in the sheaths. Thus, the use of such commercially available cannulas can ensure rapid and inexpensive regulatory clearance. One example of a known cannula device which could be used is the 0.8/25mm diameter Venflon^{RTM} sold by BOC Ohmeda AB of Helsingborg, Sweden.

In an alternative embodiment of the invention (not shown) the needles 6 can be withdrawn from the muscle or body tissue to be treated after the DNA has been injected into it and electrodes having a similar shape but made of an alternative metal such as stainless steel can be inserted before electroporation is carried out. This could be useful for example in a situation where biologically incompatible metal ions would be emitted if the needles 6 were also used as the electrodes.

The embodiments of the electroporation device described above are preferred embodiments only to which various modifications could be made. For example, the sheaths could be made of a material other than Teflon and the apertures in them could be provided in a different pattern. Further, although the device has been described as including a syringe arrangement to which the needles are connected, it will be appreciated that this need not be an integral part of the device. Thus, in an alternative embodiment, the needles in the device could be left free to be connectable to a fluid delivery system such as a syringe in use.

Consequently, the scope of the invention is not limited by the embodiments of the device as described above but rather is defined by the scope of the appended claims.

Claims

1. An electroporation device comprising: a needle for injecting a substance into body tissue; and an insulating sheath adapted to surround the needle and having one or more apertures formed along the length thereof through which an electric field may propagate in use, wherein the needle is axially moveable relative to the sheath.

2. An electroporation device as claimed in claim 1, wherein the needle for injecting a substance into body tissue also constitutes an electrode via which an electric field is propagated in use.

3. An electroporation device as claimed in claim 2, wherein the needle is connectable to a voltage source.

4. An electroporation device as claimed in claim 1, wherein the device is adapted to allow the needle to be removed from the insulating sheath after injection of the substance into the body tissue so that the needle can be replaced by an electrode rod prior to activation of an electric field.

5. An electroporation device as claimed in any preceding claim, wherein the sheath is formed from polytetrafluoroethylene.

6. An electroporation device as claimed in any preceding claim, wherein the apertures are provided along one axially extending line on the sheath only.

7. An electroporation device as claimed in any of claims 1 to 5, wherein the apertures are provided so as to be spaced around the circumference of the sheath.

8. An electroporation device as claimed in any preceding claim, wherein the apertures in the insulating sheath are formed by cutting through the sheath, pushing the apertures out or by laser ablation.

9. An electroporation device as claimed in any preceding claim, wherein two electroporation devices are coupled together.

10. An electroporation device as claimed in any of claims 1 to 8, wherein more than two electroporation devices are coupled together.

11. An electroporation device as claimed in claim 9 or 10, wherein the needles and sheaths are mounted to extend downwardly through a block in which they are arranged adjacent to one another.

12. An electroporation device as claimed in any preceding claim, wherein means are provided for sensing the depth of insertion of the needle and commencing injection of a substance via the needle when a desired depth has been reached.

13. A device comprising a needle for injection of a substance into body tissue, and means for sensing the depth of insertion of the needle and commencing injection of a substance via the needle when a desired depth has been reached.

14. An electroporation device as claimed in any preceding claim, wherein a moveable contact is provided on the device such that in use, the contact determines when the needle has been inserted to a sufficient depth into the body tissue to be treated and then causes injection of a substance via the needle to commence.

15. An electroporation device as claimed in claim 14, wherein the moveable contact further determines when the needle has been inserted to the maximum depth at which injection should be carried out and then causes injection of the substance to stop.

16. A method of electroporetic treatment of a human or non-human animal, said method comprising inserting the needle of a device according to any of claims 1 to 12, 14 and 15 into tissue in said animal, injecting an active agent through the needle into the tissue, withdrawing the needle such that the tip thereof is within the sheath, and applying an electric field between the needle and an electrode.

17. A method as claimed in claim 16, wherein the electrode is provided by the needle of a second device according to any of claims 1 to 12, 14 and 15 disposed inside a sheath.

18. A method of electroporetic treatment of a human or non-human animal, said method comprising inserting the needle of a device according to any of claims 1 to 12, 14 and 15 into tissue in the animal, injecting an active agent through the needle into the tissue, withdrawing the needle from the sheath, inserting a first electrode into the sheath such that the tip of the first electrode does not extend out of the sheath into the tissue, and applying an electric field between the first electrode and a second electrode.

19. A method as claimed in claim 18, wherein the second electrode is provided by the needle of a second device according to any of claims 1 to 12, 14 and 15 disposed inside a sheath.

20. A method as claimed in any of claims 16 to 19,

wherein the injection of the active agent into the tissue automatically commences when the device determines that the needle has reached the required depth in the tissue in the animal for injection to commence.

21. A method as claimed in any of claims 16 to 20, wherein the injection of the active agent into the tissue automatically stops when the device determines that the needle has reached the required depth in the tissue in the animal for injection to stop.

Abstract

Electroporation Device

An electroporation device is provided which includes: a needle 6 for injecting a substance into body tissue; means for generating an electric field; and an insulating sheath 14 adapted to surround the needle and having a plurality of apertures 16 formed along the length thereof through which the electric field may propagate in use, wherein the needle is axially moveable relative to the sheath. In one embodiment, the device consists of two needle and sheath assemblies mounted in a support block 4 and further includes means for sensing when the needles are at the correct depth in the body tissue to be treated to inject the substance and automatically causing injection of the substance to commence.

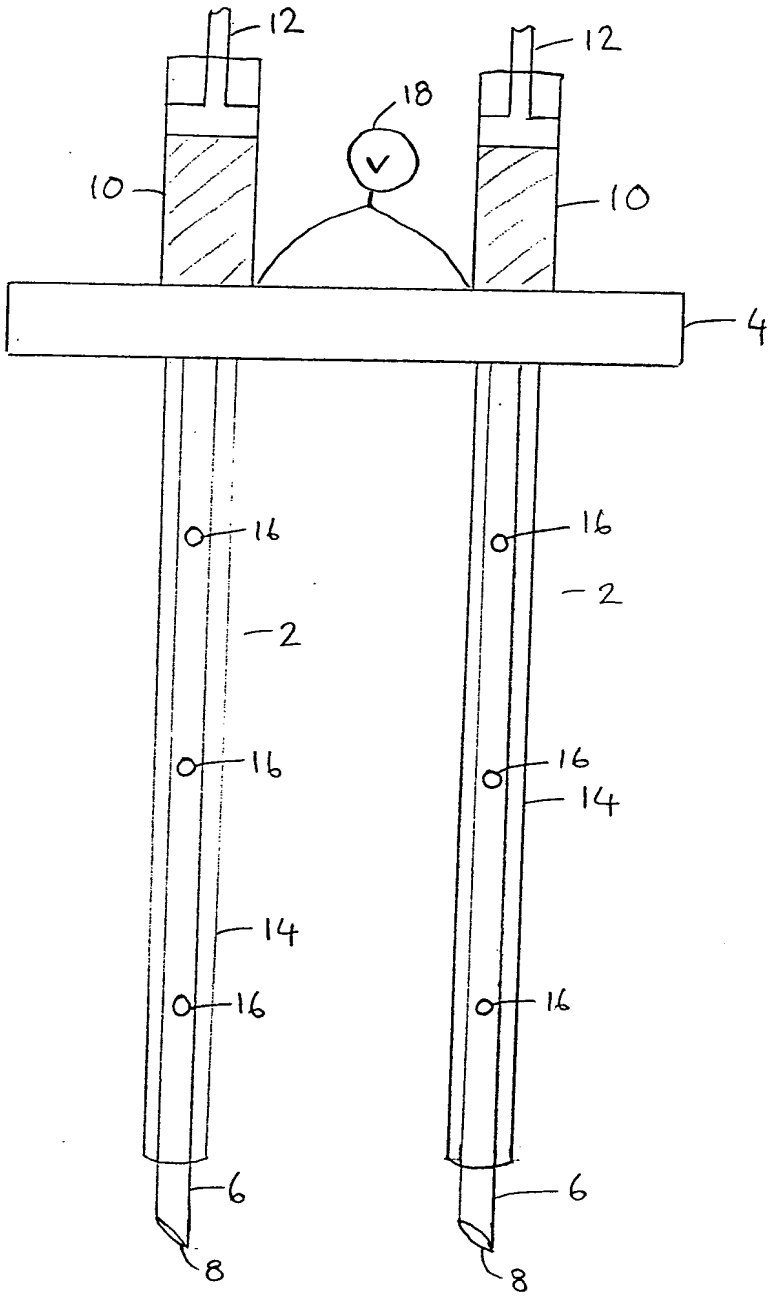

FIG. 1

FIG. 2a

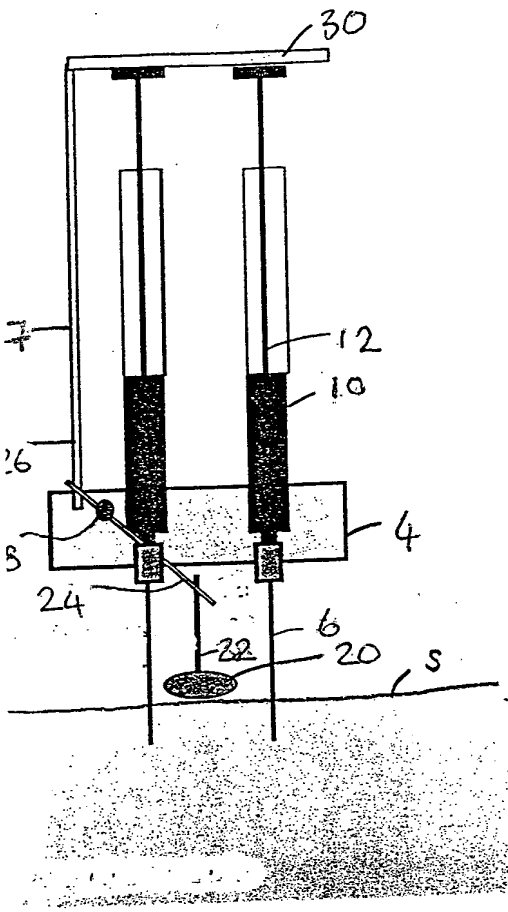

FIG. 2b

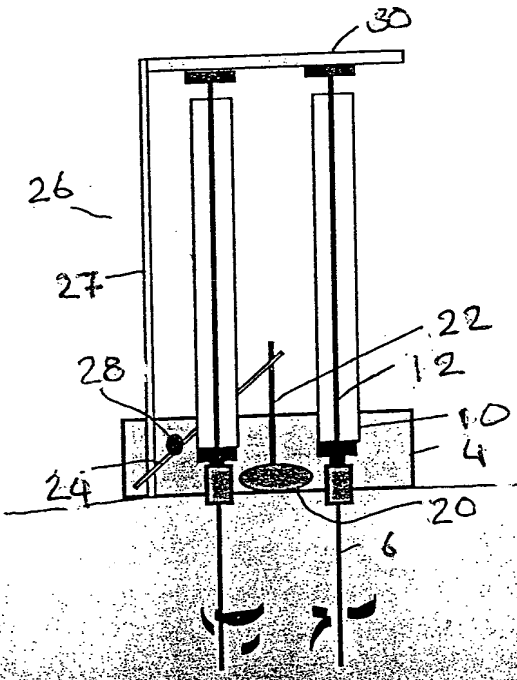

FIG. 2c